

The University of Chicago

THE INFLUENCE OF ELECTROLYTES
ON RESPIRATION IN NERVE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1932

BY
TSUNG HAN CHANG

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THE INFLUENCE OF ELECTROLYTES ON RESPIRATION IN NERVE

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Received for publication December 13, 1934

The many influences of various ions, alone and in combination, upon the colloidal structure and physiological attributes of nerve and other tissues have been exhaustively studied (see Gerard, 1932; Graham, 1933). Little has as yet been done, however, in exploring such influences on the metabolic activity of nerve. Such information would be of considerable significance in two respects. On the one hand, the pictures of nerve activity which are current coinage make rather definite statements about changes in structure and metabolism during conduction; statements which can be further checked by data upon these points. Clearly, the manner in which respiration changes in response to experimental conditions must ultimately be equated with changes in other properties to the same conditions.

Secondly, and more immediately interesting, is the question of the tie-up between metabolic and other events in nerve. The problem has been considered elsewhere: what factors fix the actual respiratory rate for a tissue, and which lead to sudden changes in it, as during activity (Gerard, 1932, 1934). The answer involves such items as the structural and chemical organization in which the oxidations proceed—colloidal dispersion, membrane permeability, ionic strength and composition, and the like.

For any integrated understanding of the chemical and physical events associated with the wave of activity in a nerve fiber, such data as we have obtained are a necessary preliminary. Part of this material has appeared in brief reports (Gerard, 1930; Chang, Gerard and Shaffer, 1932a, b); and the results show that the common tissue cations and anions do play significant rôles in regulating respiration, somewhat similar to those found for irritability, and that the mechanism of their action is, on the whole, via effects on the colloidal structure of the nerve.

METHODS. The Warburg manometric method was used throughout. Ordinarily ten manometers, eight with attached chambers of about 5 cc. volume, two of 10 cc., were used at a time. All were provided with a central cup, in which 0.1 to 0.2 cc. N/10 NaOH was placed to absorb CO₂. Some were further provided with side-arms, from which special reagents

could be tipped. In the main body of each chamber were placed about 100 mgm. of nerve in enough solution to give with the tissue 1.0 cc. (doubled in larger vessels), except for one chamber, serving as pressure and temperature control, from which tissue was omitted. The manometers were filled with air in experiments with frog nerve, with oxygen for dog nerve. The effect of a special reagent was obtained by comparing the respiration before and after tipping the reagent from the side-arm into the main chamber, or by comparing the respiration of matched nerves (from the same animals) with and without the reagent added to the main solution. In a few cases results from different individual frogs were compared. Oxygen consumption is expressed throughout as a rate, (Q_{O_2}), cubic millimeters of oxygen consumed per hour per gram of fresh tissue.

Using pure isotonic saline (or Ringer) as the basic solution, all others are expressed as isotonic percentage of each salt added. Thus "10 per cent KCl" means 10 parts isotonic KCl to 90 parts isotonic NaCl (or Ringer). Solutions were freshly prepared by mixing stock solutions, and were brought to the desired pH (usually 7.4) with Sorenson's phosphate buffer (1:10) or by direct titration. Final pH was checked with the quinhydrone electrode.

Nerves were rapidly dissected after pithing and bleeding the green frogs, two being ordinarily used per chamber. They were placed so that two chambers each contained one nerve of the pair from each frog. Most of the experiments on frog sciatics were performed at 21°C., a few at 25°C., and some at 37°C.; all those on dog vagus at 37–38°C. It is important to note, in evaluating respiration results, that nerves from the same frog may differ by 10 per cent, from different ones by 100 per cent, under the same conditions. Of course, consistent differences of even less than 10 per cent become significant. All statements of fact in this report are based on several consistent (at least three) duplicate experiments, though in some cases only one is given in the tables. In all, about one thousand runs were made.

RESULTS. A. *The effect of alkali-salts.* 1. *NaCl.* It has long been known that the frog sartorius will twitch when soaked in isotonic NaCl solution. Riggs (1919) claimed that frog nerves produced an extra amount of CO_2 under such treatment, but his actual data were not convincing. Gerard (1930), using the Warburg technique, did not find a corresponding increase in oxygen consumption. As NaCl is present in sea water and all biological fluids to over 80 per cent of the total salt concentration, it was important to make this point certain. The results of several series of experiments warrant the conclusion that at 20 to 25°C. isotonic NaCl has no definite effect on nerve respiration, not over a 5 per cent increase at most and irrespective of whether or not the nerve sheath is split to insure rapid penetration. At 37–38°C., however, the effect is very marked (see table 1).

At 37° both frog and dog nerve show an increase in oxygen consumption in NaCl as compared to Ringer. (No phosphate buffer was added to the Ringer in these experiments, since at the desired pH much of the Ca^{++} in the solution, 0.024 per cent CaCl_2 , or tissue would be precipitated by the anions. Ringer with phosphate added acts about the same as pure saline, the effects of which, therefore, are largely related to calcium lack.) The values in saline are regularly high from the start (by $\frac{1}{4}$ to $\frac{1}{3}$), but become strikingly higher after four or five hours, when the tissue exhibits a great increase in oxygen consumption. In Ringer this increase is less and occurs two or three hours later than in saline. This late increase in respiration is not due, as we first believed, to a form of ionic and osmotic injury to the tissue, exaggerated by the low calcium and consequent greater permeability of cell membranes, but to bacterial growth. This question is discussed more fully in the following paper, but it should be pointed out here that the earlier interpretation may be in part correct. The increase

TABLE 1

NERVE	TEMPERATURE	TIME	QO_2	
			Ringer	NaCl
		hours		
Frog, intact (2 exp.).....	21°	20	40	43
Frog, split (3 exp.).....	25°	7	53	55
Frog, intact (3 exp.).....	38°	4	94	96
		Next 5	44	182
Dog vagus (3 exp.).....	37°	5	143	308
		Next 4	243	372

in oxygen consumption due to bacterial growth is definitely modified, in time and quantity, by the presence or absence of calcium ion. This is no longer true when the nerve is killed by boiling at the start of the run—in this case the bacterial rise is much accelerated and calcium has no influence. The calcium, then, appears to determine the speed with which the nerve disintegrates, gives off nutrient substances or permits bacteria to invade it, rather than directly affecting bacterial growth. The action is, thus, in the usual direction—the cell integrity being maintained longest in balanced salts, diffusion increasing in calcium lack, and a complete disintegration resulting from thermal death. The influence of sodium, per se, will be brought out in connection with the use of non-electrolyte solutions.

2. *Potassium salts.* Riggs (1919), Gerard (1930) and Schmitt (1931) have reported that the respiration of frog sciatic nerve is depressed about 50 per cent by isotonic KCl. Gerard also used 10 per cent isotonic KCl in saline, and found no effect. The present results are in accord, and we

have further determined the potassium effect over the whole range of concentration. Average results, shown in the accompanying graph (fig. 1) indicate a practically maximal effect at 50 per cent KCl. After being kept in Ringer in the ice box for 18 hours, following a period in potassium mixture, all the nerves showed good action potentials. Also, respiration again increases in Ringer, so the potassium depression is clearly reversible. The concentration required to produce a marked effect on respiration is higher than that which is required to markedly decrease irritability. The latter, according to Misske (1930), is from 5 to 7 per cent, while a 10 per cent solution is not sufficient to cause a detectable depression in oxygen consumption.

A similar effect of K^+ was also found with dog vagus, an average depression of 68 per cent being obtained in isotonic KCl. For muscle (frog

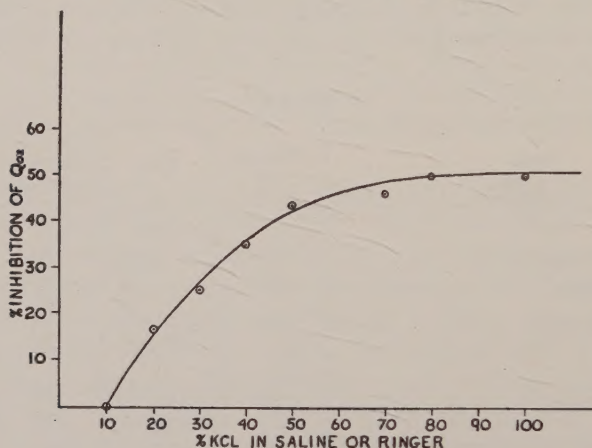


Fig. 1

sartorius) the case is very different. In confirmation of Fenn (1931), we have found that an excess of potassium increases respiration. Pure isotonic KCl solution leads to increases of about two-thirds above the value in Ringer (Q_{O_2} of fifty experiments in Ringer = 38), and even the addition of 30 per cent of this solution causes a distinct increase.

3. *Rhubidium and caesium*. Both these ions depress nerve respiration, though not so much as potassium. In 50 per cent isotonic solution (with sodium) there is little effect; in pure isotonic solution of their chlorides, each gives a 25 per cent inhibition.

The respiration of muscle is likewise depressed by these ions, especially caesium, despite a vigorous twitching. This is in contrast to the increase associated with potassium contracture.

B. *The effect of alkaline earths*. 1. *Calcium*. The maximal depressant

effect of this electrolyte on the Q_{O_2} of the frog's sciatic, as previously reported (Gerard 1930), is about the same as that of KCl (50 per cent.) Its effect on the dog's vagus is somewhat less (32 per cent). When graded concentrations are used, the maximal depression is obtained with 80 to 100 per cent isotonic $CaCl_2$. In many experiments, no change resulted from 50 per cent solutions and only a 30 per cent depression appeared with

TABLE 2

SOLUTION	ISOTONIC	INCREASE IN Q_{O_2}	NUMBER OF EXPTS.	SOLUBILITY (Ca^{++}) IN H_2O AT $25^\circ C.$, MGM. PER CENT Ca^{++}
	<i>per cent</i>	<i>per cent</i>		
NaOxalate				0.2
	20	74	2	
	30	90	2	
	100	88	2	
NaCitrate				0.8*
	0.5-2	9	2	
	5	19	1	
	10	54	5	
	20	52	2	
	35	107	3	
	50	82	3	
	100	76	11	
NaFluoride				0.9
	30	25	3	
NaTartrate				8.0
	10	0	1	
	20	9	1	
	35	24	2	
NaPhosphate (buffer, pH 7.8)				10
	10	15	15	
	100	40	50	
NaSulphate	100	7	8	53

* Estimated from data of McLean and Hastings, 1933.

80 per cent solutions; in others even 50 per cent solutions gave nearly maximal depressions. Misske (1930) obtained a marked decrease in irritability with 25 per cent $CaCl_2$ in saline but not with 10 per cent. Hence the effect on respiration is again less than that on irritability. The respiration of brain slices (dog and rabbit, 38°) is also depressed by excess calcium.

1a. *The effect of decalcifying salts.* It is clear that excess of free Ca^{++}

in the medium tends to lower respiration, as well as irritability, and results in pure NaCl suggest the converse. The Ca content of nerve is higher than that of the blood or lymph which bathe it —0.13 to 1.2 per cent of the fresh weight of rabbit sciatic (Kraus, etc., 1924); 0.6 to 1.5 per cent of frog (Simon and Szelöczey, 1928; Fenn, 1934, gives the more probable figure 0.025—still well over 0.01, for serum). Decalcifying salts, by rapidly reducing the Ca^{++} concentration inside the axones themselves as well as in the outside medium, serve to extend the results obtained by simply omitting calcium from the medium. The effect varies with the nature and amount of active anion added. All such anions lead to an immediate increase of respiration even at 21°C. and, especially interesting, the increase tends to vary inversely as the free Ca^{++} concentration that remains in each case.

TABLE 3

SOLUTION	QO_2 (8 HOURS)	NUMBER OF, EXPTS.
CaCl_2 8	33	(3)
NaCl 1		
CaCl_2 8	32	(3)
NaCitrate 1		
NaCl	50	(1)
NaOxalate 1	71	(2)
NaCl 1		
NaOxalate 1	77	(2)
NaCitrate 1		

TABLE 4

SOLUTION	QO ₂ FOR 6 HOURS	DECREASE
		<i>per cent</i>
Ringer.....	33.0	
CaCl ₂ (2)		
KCl (8)	18.7	43
Ringer.....	34.6	
CaCl ₂ (5)		
KCl (5)	13.4	61

With Na citrate (23 experiments and controls) below 30 per cent isotonic in Ringer or saline, the effect is present but not definitely maximal. Even 1 per cent citrate, however, gives a 10 per cent increase. At 30 per cent or above, a maximal increase is obtained, from 70 to over 100 per cent. The results with various anions are briefly summarized in table 2.

Since anions in general tend to increase respiration, permeability, etc., the effect increasing with valence (Mathews, 1904; Lucké and McCutcheon, 1932), the citrate ion might act in these experiments by its peptizing effect on negative or its coagulating effect on positive colloids, aside from any relation to calcium. The following tests, however, demonstrate that such an effect, if present, was very small. In one series, citrate was added, or not, to an excess of calcium. Its presence was immaterial, so at least it is not effective as a complex (table 3). Another point, 10 to 20 per cent isotonic citrate produces as marked an effect as any higher concentration. This would combine with all the calcium ion (0.004 M)

and should therefore be a maximal concentration on this basis; whereas stronger solutions might be expected to produce greater effects if dependent on colloidal changes (see table 2). Finally, when respiration is increased by addition of oxalate, the further addition of citrate has little effect (table 3). This would be expected if calcium removal is the critical point, not if anion charge is, for, of course, the citrate ion is trivalent, oxalate only bivalent.

As already noted (table 2), oxalate alone gives about the same increase in respiration (60–90 per cent) as does citrate alone. The respiration increase in Na tartrate (about 25 per cent) or Na fluoride (25 per cent), moreover, is definitely less than that in oxalate or citrate, in harmony with the higher free Ca^{++} they permit, though phosphate gives an intermediate effect. (A particular, depressive action of fluoride may also enter into the action of this ion. See, e.g., Dickens and Simer, 1929.) Though Riggs (1919) has emphasized the stimulating effect of sodium sulphate, one would predict in terms of decalcification that, because of the solubility of CaSO_4 , sulphate could not be very effective in augmenting nerve respiration. In the present experiments with isotonic sodium sulphate at 20°C ., the respiration of frog's sciatic nerve increased under 10 per cent in 7 to 9 hours (table 2), as compared to NaCl controls; nor did splitting the nerve sheath alter the results. During a further 10 to 12 hours the results were irregular, fluctuating about normal by 10 to 30 per cent. Sulphate, therefore, exerts at best a small positive effect, in harmony with the relatively great solubility of its Ca salt.

1b. *Calcium and potassium.* Ringer with the calcium removed behaves like isotonic NaCl, so that the small potassium concentration of the former appears to be without influence on the calcium action. In view of the marked antagonism between Ca^{++} and K^+ on many properties of nerve, it seemed desirable to explore rather fully their combined effects on respiration. Direct combination of both ions, in excess concentration, does not lessen the independent depressive action of either one alone (table 4).

Menten (1912) reported that potassium salts whose anions remove calcium have no depressive effect when injected into various mammals, which would speak for synergy rather than antagonism. Experiments performed on isolated nerve with potassium salts of these anions largely agree (table 5). The depressant action of K^+ in potassium citrate and KH_2PO_4 was as marked as in KCl, but recovery on placing the nerves in Ringer was better after these than after the chloride. When, further, KCl and sodium citrate were mixed in varying ratios, the depressant effect was much lower in mixtures rich in citrate than in pure KCl, even allowing for dilution of the K^+ (table 6). These experiments demonstrate an antagonism between potassium and citrate, probably primarily via calcium ion changes. But since 10 per cent citrate should suffice to give a maximal decalcifying

effect, the further increase of citrate action with increasing concentration suggests, also, a direct antagonism between the cation potassium and the anion citrate.

1c. *Calcium on dog vagus*. The dog vagus even more than the frog sciatic, at 38°, behaves quite differently in normal Ringer and in decalcified Ringer. In the latter, the Q_{O_2} is considerably higher from the start and at the 3rd to 5th hour a further rise is nearly always seen. A rise in ordinary Ringer also often appears, but it is small and much delayed. In serum, the initial respiratory rate is high and no late rise occurs (see the following paper), but addition of sufficient Ca^{++} remover causes a further increase in oxygen consumption (table 7).

Muscle respiration is similarly increased by addition of decalcifying salts, up to 200 per cent; and the increase evoked by potassium is also

TABLE 5

SOLUTION	Q_{O_2} (FOR 4 HOURS)	Q_{O_2} (FOR 3 HOURS) AFTER PLACING IN RINGER	INCREASE AFTER PLACING IN RINGER
			per cent
KCl.....	13.2	18.8	42
K-citrate.....	16.2	28.4	75
KCl.....	23.3	26.7	15
K_2HPO_4	22.0	37.2	69

TABLE 6

SOLUTION	Q_{O_2} IN 10 HOURS	DEPRESSION IN KCl OR KCl-CITRATE MIXTURE
NaCl.....	39.2	
KCl (60).....	33.8	14
NaCitrate (40).....		
NaCl.....	33.4	
KCl (80).....	24.3	27
NaCitrate (20).....		
KCl.....	16.2	55
KCl (90).....	25.7	29
NaCitrate (10).....		

augmented by these anions, up to twice that due to potassium alone. Calcium and potassium together give smaller increases than potassium alone, and excess calcium leads, often after an initial increase, to a marked falling off in respiration.

2. *Magnesium*. $MgCl_2$, used in amount equivalent to the $CaCl_2$, has practically the same effect on nerve respiration and seems fully able to replace this ion. In excess of magnesium, respiration is depressed 45 per cent. It is noteworthy that this equivalence does not extend to the grey matter of the brain, magnesium ion appearing indifferent to the respiration of this tissue despite its well known "anesthetic" action. In eleven experiments on dog or rabbit brain, the Q_{O_2} for the first hour or two averaged 950. This fell in the fourth hour by 27 per cent with no addition, by 28 per cent after addition of magnesium (to 0.03 M), and by 48 per cent

after addition of a similar amount of calcium salt. (It is interesting that the central nervous system is especially sensitive to low calcium. Flushing the dorsal cavity with NaCl, let alone the use of Ca removers, gives convulsions in cats. (Weed and Wegeforth, 1916.)

3. *Barium*. The stimulating action of this salt on muscle has long been known, and powerful twitchings have been obtained with as little as M/30,000 BaCl₂ in saline (Chao, 1934). On nerve respiration its action, like the other alkaline earths, is depressive, giving in 35 per cent isotonic solution (0.03 M) a decrease of 30 to 50 per cent, which progresses in

TABLE 7
Dog vagus and sciatic, 37°C.

SOLUTION	NUM- BER OF EXPTS.	DURA- TION	QO ₂	CHANGE
		hours		per cent
Serum 9	3	8	189	
NaCl 1				
Serum 9	3	8	192	
Na-KPO ₄ 1				
Serum	3	12	156	+13
Serum 9	3	12	177	
NaCitrate 1				
Serum 7	3	4	157	
NaCl 3				
Serum 7	3	4	224	+45
NaCitrate 3				
Serum 7	3	8	212	
NaCl 3				
Serum 7	3	8	275	+30
NaOxalate 3				

TABLE 8

SOLUTION	QO ₂ (6 HOURS)	DECREASE
		per cent
NaCl	36.0	60
M/50 AlCl ₃ in NaCl	14.2	
NaCl	41.0	59
M/100 AlCl ₃ in NaCl	16.7	
NaCl	35.0	62
M/200 AlCl ₃ in NaCl	13.3	

later hours to 60 or 75 per cent. The effect of BaCl₂ on grey matter is similar: concentrations from 0.5 to 10 M give a decrease of about 10 per cent.

C. *The effect of a trivalent cation (AlCl₃)*. The powerful coagulating effect of aluminium salts (probably related to the precipitating action on negative colloids) was at once apparent from the appearance of nerves immersed in dilute AlCl₃. The depressant action on respiration was marked at much lower concentrations than in the case of the alkaline-earth. A maximal decrease (60 per cent) was obtained at concentrations

as low as $M/200$, and probably this was still considerably above that required for a maximal effect (table 8). A somewhat smaller decrease (40 per cent) was obtained in two experiments on dog's vagus.

It is worth noting that citrate which, presumably, disperses colloids, increases the weight of nerves immersed in it, while aluminium, with the reverse effect, decreases nerve weight.

D. *The effects of monovalent anions.* Mathews (1904) maintained that the effect of any given salt depends on the balance of opposed effects of the contained cation and anion, a view that has received strong support in the experiments of Lucké and McCutcheon on the cobaltamines (1932). Both ions are surely concerned in producing effects on structural colloids. For nerve, Netter (1927) found the axiolemma impermeable to anions, which also have little effect on the resting potential. Höber and Strohe (1929) found on frog's sciatics that an electrotonic current decreased slowly after a brief rise in NaCl, rapidly in CNS^- or I^- ; NO_3^- and Br^- acted like Cl^- ; SO_4^- had no effect; and the addition of 0.02 per cent CaCl_2 to any of these rendered its effect negligible. They also found that the presence of CaCl_2 largely cancels the action of the same salt series on nerve irritability. Under such conditions, I^- , Br^- , NO_3^- and SO_4^- caused a very slight increase followed by a negligible drop, CNS^- an immediate fall or no change for some time.

In harmony with the above, we have found the respiration of nerve largely indifferent to these anions. This is true for the frog's nerve (at $21^\circ\text{C}.$) as well as the dog's (at $38^\circ\text{C}.$). NaSCN and especially NaNO_3 have, however, a distinct effect (table 9).

On muscle, these anions have greater effects, leading to increases up to 400 per cent. The order of diminishing effectiveness is: CNS , I , SO_4 , Br , NO_3 , Cl .

E. *The effect of isotonic non-electrolyte (cane sugar).* In association with the loss of irritability of muscle in sugar solution, Fenn (1931) found a marked increase in respiration. Nerve loses its irritability more slowly in sugar solution, though splitting the sheath hastens this considerably (Feng and Gerard 1930), and respiration is depressed rather than increased. Also, the respiration changes much sooner in intact nerve than does the irritability. A depression of 10 to 45 per cent (average 26 per cent) is usually present within one to two hours, and is then maintained or increased for over twenty hours (21 – $25^\circ\text{C}.$, frog's sciatic). Splitting the sheath has no effect on the speed of action on respiration. Dog vagus shows a similar but smaller depression, 22 per cent in two experiments. After exposure to the sugar solution, nerves become translucent and rather stiff.

E1. *Sugar and salt mixtures.* For muscles, the loss of irritability and the increased respiration in sugar solution are reversible phenomena, being

restored to the original level when the tissue is transferred into saline or Ringer (Overton, 1904; Fenn, 1931). The loss of irritability of nerve in sugar solution is also reversible, and hence is presumably due to the diffusion out of electrolytes.

In accord with expectations, addition of NaCl to the sugar largely prevents the fall in respiration. Addition of KCl or CaCl₂, on the contrary, does not restore the Q_{O_2} , but may further depress it. Ten to 30 per cent by volume of NaCl in sugar solution has but little effect on the initial sugar

TABLE 9
Influence of anions (lytropic series)
Frog sciatic (21°C.)

SOLUTION	Q_{O_2}	DURATION	INCREASE
		hours	per cent
NaCl.....	39.5	5	
NaBr.....	39.6	5	—
NaCl.....	46.6	7	
NaI.....	48.8	7	—
NaCl.....	40.6	8	
NaNO ₃	50.0	8	23
NaCl.....	42.0	8	
NaCNS.....	44.7	8	+

Dog vagus (37.5°C.)		
	Q_{O_2} FOR 4 HOURS	INCREASE
		per cent
Ringer.....	223	
NaBr.....	225	
NaI.....	227	
NaNO ₃	246	10
NaCNS.....	271	21

depression, but when a 40 to 60 per cent solution is used, this depressant effect is largely removed. Later, even 20 per cent NaCl is effective in preventing the further fall in pure sugar; and the respiration in 40 per cent NaCl is usually 20 to 30 per cent higher than in pure saline. The presence of ions (NaCl) is clearly essential to the respiration of nerve. NaCl likewise restores in an hour the normal opacity to a nerve rendered translucent in sugar. The stiffness, however, persists after 13 hours.

In sharp contrast to the findings on muscle, addition of KCl to sugar fails to restore the normal respiration, but their depressive effects sum

(table 11). The same was found for calcium in a single experiment (1 calcium to 3 sugar cut the Q_{O_2} 30 per cent) and depressions are progressive with time.

Extending previous observations, in five experiments with nerves in NaCl and glucose, addition of insulin (10^{-5} to 10^{-1} units) had no effect on respiration. Glucose alone also has no influence in moderate concentration.

F. *Weight changes.* Isolated tissues are not in equilibrium with isotonic saline solutions, and it is interesting to note the influence of various

TABLE 10
Frog sciatic (25°C.)

SOLUTION	EXPTS. NO.	DURATION	Q_{O_2}	CHANGE
		hours		per cent
Ringer.....	3	7	51.6	
M/4 sucrose.....	3		40.9	-21
NaCl*.....	3	20	31.5	
M/4 sucrose*.....	3		18.0	-43
NaCl.....	15		42	
M/4 sucrose.....	11		31	-26

* Frogs long in captivity.

TABLE 11
Frog sciatic, 20°C. 7 to 20 hours. Q_{O_2} in Sucrose = 28

Solution.....	Sucrose	10	9	8	7	6	0
	NaCl	0	1	2	3	4	10
Q_{O_2}		100%	100+%	125%	140%	150%	160%
Solution.....	Sucrose	10	7	3			
	KCl	0	3	7			
Q_{O_2}		100%	80%	60%			

ions on the swelling of nerves, though no systematic study has been made. Frog nerve, at 20°, gains about 8 per cent in weight during 1 to 3 hours in frog Ringer. Even in mammalian Ringer there is a slight gain, about 5 per cent, despite its hypertonicity. In dog serum, frog nerves maintain their initial weight for days. Pure isotonic potassium chloride leads to enormous swelling, up to doubling in thirty hours, which is largely reversed by subsequent treatment with NaCl or Ringer. Weight increases of 20 per cent follow immersion in NaCl for 10 or more hours. In isotonic calcium solutions there is little weight change for 10 hours, followed by swelling to about 20 per cent increase in another twelve hours (fig. 2).

Pure lithium or caesium acts like Ringer, giving but small weight increases. Swelling in sucrose is about the same as in NaCl, as it is also in the following sodium salts: iodide, thiocyanate, tartrate and citrate. Bromide and nitrate and possibly sulphate give less swelling than the chloride. Further data on dog nerves in saline and protein solutions are presented and all discussed in the following paper.

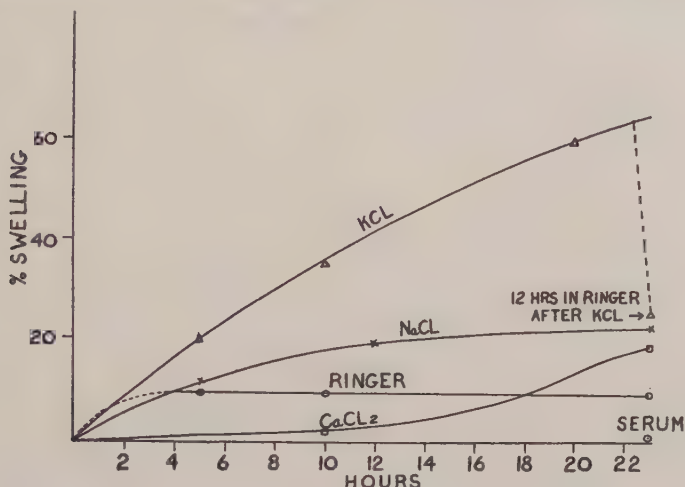


Fig. 2

DISCUSSION. In most experiments supporting the existence of a stimulating effect of sodium chloride, pure isotonic solution has been used. Apparently the out-diffusion of Ca^{++} from the plasma membrane or the interior of the cell has not always been considered. But Ca-lack is far more effective in causing dispersion of structural colloids and in increasing permeability than is Na-excess. At lower temperatures this process seems to be exceedingly slow in nerves, each fiber being wrapped in a myelin sheath and neurilemma. This may explain the absence of any measurable effect on nerve respiration in isotonic NaCl at 21°C. in a period of twenty hours. At higher temperatures (37–38°C.) the changes are greatly accelerated, so that in saline the respiration of the nerve is higher at the start and rises further, as a rule in 3 to 5 hours, than when in Ringer.

The important rôle of NaCl in nerve is shown by the effects of its absence or deficiency rather than by its excess. Thus, in sugar solution, as soon as the major amount of this salt is removed from the interstitial fluid or the plasma membrane, the oxygen consumption is decreased. The respiration decrease occurs long before irritability loss, to which it may contribute. A certain amount of the NaCl in the medium is necessary to maintain the normal respiration as well as irritability of nerve.

The effect of KCl on nerve respiration is atypical, for its action on structural colloids, like that of Ca-lack (Höber and Strohe, 1929), is to increase dispersion and so the permeability of the tissues. Also potassium excess acts, like cathodal polarization (Woronzow, 1925; Mackuth, 1926; Graham, 1933), to give a temporary increase followed by a decrease of resting potential, and electrotonic current spread. Irritability, though ultimately depressed, is for some time definitely raised—especially to long current pulses (Blumenfeldt, 1925; Höber and Strohe, 1929). But respiration is uniformly and permanently depressed, no temporary rise having appeared. (A brief effect at the start would, however, be missed.) Similarly, potassium somewhat diminishes the spike action potential and strongly the after-potential; prolongs absolutely and relatively refractory periods, and slows conduction (Graham, 1933). Graham gives no indication of early changes in the opposed direction, though occasionally a long after-potential accompanies potassium treatment. KCl is effective on nerve respiration only in excess; its absence apparently has no effect. Any complete removal, however, as of calcium by citrate, was not obtained. The oxygen consumption of skeletal muscle, on the contrary, is greatly increased by KCl (Fenn, 1931; Hegnauer, 1933). This is most probably due to the contracture induced by this salt, and the fundamental effect may be a depression. This is at least the case for heart muscle, the respiration (and tone) of which is decreased by excess potassium (Arnoldi, 1924; Victor, 1933), as in nerve.

CaCl_2 exerts an effect in both excess and deficiency. Its absence or decrease leads to a rise in oxygen consumption; its excess to a fall. These changes parallel reasonably those of irritability, etc., as well as the clinical manifestations associated with hypo- or hypercalcemia. Here again the respiration of heart is affected as is nerve, that of skeletal muscle inversely.

The more prompt and intense change in respiration obtained on addition of decalcifying agents, as compared with simple absence of Ca from the medium, may be interpreted as the difference between active and passive removal. As the decalcifying ions penetrate, Ca^{++} is locally reduced to very low concentrations, permeability is heightened, and further penetration follows. A rapid invasion of the axone and prompt loss of Ca^{++} results.

On the whole, the effects of ions on nerve respiration can be interpreted in terms of their effects on the colloidal structure of the nerve. The cations lessen colloidal dispersion, decrease enzyme surface activity, and depress respiration. The anions evoke opposite colloidal and respiration changes. In addition, specific ion effects, as for calcium, often play a primary rôle.

Of all nerve properties so far studied, the only ones changing from the start in the same direction when acted on by excess of either potassium or

calcium are conduction velocity, respiration and, possibly, spike height. There is obviously little ground, on the basis of the available data on salt action, for drawing extensive conclusions as to the interrelations of the phenomena attendant on nerve function. The tedious exploration of the action of sodium, calcium and potassium ions, in varying amounts and ratios on most nerve properties still awaits adequate performance. There is, however, no question that the amount of these ions and their distribution between the inside and outside of cells play a predominant rôle in controlling nerve conduction, sense organ discharge, and reflex activity. (Gerard, 1932; Feng, 1933; Talaat, 1933; Huggins and Hastings, 1933; Boyd and Ets, 1934; Cowan, 1934; etc.)

SUMMARY AND CONCLUSIONS

1. The influence of several common ions on nerve respiration has been investigated. In general their action on respiration is closely related to their effect on the colloidal structure of nerve.

2. Sodium is important in maintaining the normal oxygen consumption of nerve. In its absence respiration is decreased 10 to 40 per cent, a stimulating effect with excess is doubtful.

3. Potassium manifests a depressant action only in excess (maximal depression 50 per cent at 50 per cent solution). Its absence has no effect. Such a depressant effect on respiration is not parallel to irritability and some other changes in nerve induced by potassium.

4. Calcium is especially important since small increases or decreases in its concentration have a marked influence on nerve respiration and irritability. Respiration, like irritability, varies inversely with calcium content.

5. Magnesium, but not barium, is able to substitute for calcium in nerve; neither can in grey matter.

6. All the decalcifying sodium salts increase nerve respiration, the increase roughly paralleling decalcifying power, from 25 per cent with tartrate and fluoride to about 100 per cent with oxalate and citrate.

7. Aluminium, the only trivalent cation studied, depresses nerve respiration and has a strong coagulating effect.

8. Most monovalent anions are of little or no influence, possibly related to their inability to penetrate the nerve membrane.

9. Isotonic sugar solution depresses nerve respiration to 50 per cent. Addition of NaCl restores it to normal, while addition of KCl or CaCl₂ further depresses, thus giving additive effects with the sugar.

10. No antagonism on nerve respiration is manifest between sodium and calcium or potassium and calcium, but cations and anions do antagonize.

11. The results favor the common view that a structural colloid of nerve is negatively charged.

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